Postmortem Proteolysis of Breast and Leg Muscles from Taiwan Colored Chickens and Silkie Bantams

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ABSTRACT : Postmortem proteolysis of breast (BM) and leg (LM) muscles from Taiwan colored chickens (TCC) and silkie bantams (SB) at 5°C were compared. Myofibrils were prepared from BM and LM samples that were randomly taken from the carcasses of SB and TCC after 0, 1, 3, 7 and 14 days of storage at 5°C. pH of samples was determined, and degradation of myofibrillar proteins was analyzed by the SDS-PAGE and western blots. The results showed that pH was lower in BM than in LM samples from both avian strains. Appearance of 30 kDa components and disappearance of titin and nebulin were more rapidly as seen on SDS-PAGE in BM than in LM samples. Western blots labeled with a monoclonal antibody to desmin also demonstrated that desmin degraded more quickly in BM samples. Our data might suggest that postmortem proteolysis occurred more rapidly in BM than in LM from both TCC and SB. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 5 : 739-743)***

Key Words : Taiwan Colored Chickens, Silky Bantam, Postmortem Proteolysis

INTRODUCTION

The postmortem degradation of skeletal muscle proteins at refrigerated temperature and its relationship to meat tenderness have been extensively studied (Koohmaraie, 1992, 1994; Taylor et al., 1995). It is generally believed that the calpain system plays a major role in this post mortem degradation (Koohmaraie et al., 1987; Boehm et al., 1998). Earlier studies (Hay et al., 1973) have reported that postmortem storage results in appearance of several polypeptide fragments migrating in the 30,000-Da range in SDS-PAGE. It has now been known that the ~30 kDa polypeptides were resulted from degradation of troponin-T in beef muscle (Ho et al., 1994; Muroya et al., 2003, 2004), and that density of the 30 kDa band was correlated to ultimate tenderness (McBride and Parrish, 1977). In addition, titin, nebulin and desmin all are filamentous proteins in myofibrils and are very susceptible to postmortem degradation (for reviews, see Robson et al., 1997). Taiwan colored chickens (TCC) and silkie bantam (SB) are two popular poultry meat sources in Taiwan. Much less information, however, is available regarding postmortem changes in the muscles of TCC and SB. The purpose of this study, therefore, was to compare the postmortem proteolysis of breast and leg muscles with those two strains. The changes in pH and degradation of myofibrillar proteins were examined.

MATERIALS AND METHODS

Sample preparation

Taiwan colored chickens (an average live weight of 2.9 kg) and silkie bantam (an average live weight of 2.7 kg) were slaughtered in 16 weeks old by using normal commercial practices in a local plant. The carcasses were vacuum-packed and stored at 5°C. Breast (BM) and leg (LM) muscles were randomly sampled at 1, 3, 7 and 14 days of storage. The BM and LM (~4 h postmortem) taken from the carcasses right after the slaughter process were used as the 0-day samples. This experiment was done with three repeats. Fifteen birds randomly selected from each strain were used for one repeat and three birds for each time period. After sampling, BM and LM muscles were ground through a 3 mm plate with a common grinder (Model TS-32, Tritacarne Company, Italy) and evenly mixed for pH measurements and myofibril purification. pH measurements of BM and LM were done by the method of Farouk and Swan (1997). Statistical analysis was done by using the GLM (General Linear Model) procedure of SAS (SAS Institute Inc., 1986).

SDS-PAGE analysis

BM and LM myofibrils were purified via the method of Huff-Lonergan et al. (1995). The protein concentration of the myofibril samples was determined using a modified biuret method (Robson et al., 1968). The myofibril samples (4 mg/ml) for SDS-PAGE were mixed with equal volume of the sample buffer to give a final concentration of 2 mg/ml by the method of Wang et al. (1988). The same amount of protein (150 μg) from each myofibril sample was loaded into each well of the gels. The proteins migrating below the myosin heavy chains were routinely analyzed in a 12% tris-glycine slab gel (acrylamide:methylenebisacrylamide was 37.5:1, w/w) according to the method of Laemmli (1970). Degradation of titin and nebulin was examined in an 8% tris-glycine slab gel (acrylamide: methylenebisacrylamide

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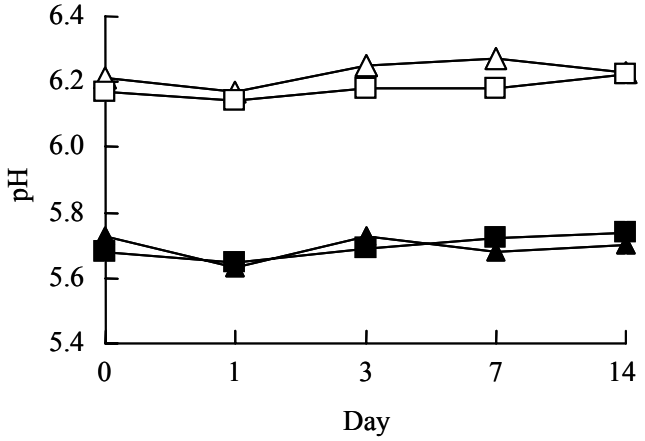

Figure 1. Changes in pH of breast (BM) and leg (LM) muscles from Taiwan colored chickens (TCC) and silkie bantam (SB) during postmortem storage at 5°C. Standard error of means (S. E. M.) = 0.07. ■, BM samples of TCC; □, LM samples of TCC; ▲, BM samples of SB; Δ, LM samples of SB.

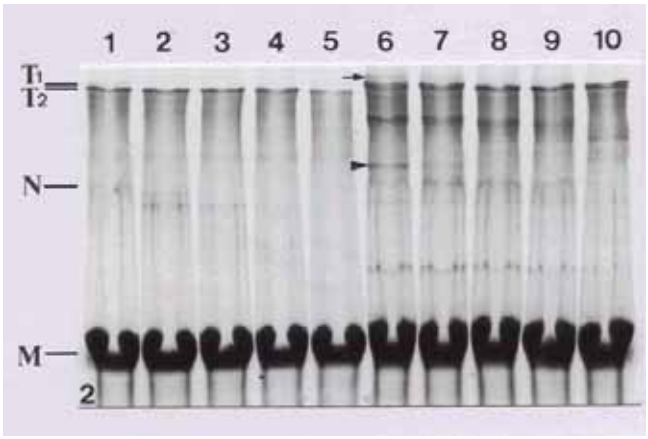

Figure 2. Changes in titin and nebulin of breast (BM) and leg (LM) muscles from Taiwan colored chickens. BM samples = lanes 1-5; LM samples = lanes 6-10; 0-day = lanes 1 and 6; 1-day = lanes 2 and 7; 3-day = lanes 3 and 8; 7-day = lanes 4 and 9; 14-day = lanes 5 and 10. T1, titin 1; T2, titin 2; N, nebulin; M, myosin heavy chains. Arrow: Titin 1 in LM samples. Arrowhead: Nebulin in LM samples.

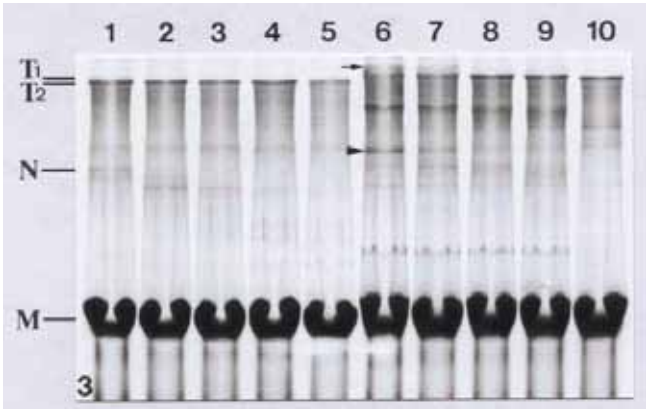

Figure 3. Changes in titin and nebulin of breast (BM) and leg (LM) muscles from silkie bantam. BM samples = lanes 1-5; LM samples = lanes 6-10; 0-day = lanes 1 and 6; 1-day = lanes 2 and 7; 3-day = lanes 3 and 8; 7-day = lanes 4 and 9; 14-day = lanes 5 and 10. T1, titin 1; T2, titin 2; N, nebulin; M, myosin heavy chains. Arrow: Titin 1 in LM samples. Arrowhead: Nebulin in LM samples.

was 200:1, w/w) (Wang et al., 1988).

All gels were run at 15 mA at 25°C. A SE 400 slab gel electrophoresis unit (Hoefer Scientific Instrument, San Francisco, CA) was used. Gels were stained in a solution of 0.05% (w/v) Coomassie blue R-250, 45% (v/v) methanol and 9.2% (v/v) acetic acid for 4 h and destained in 10% (v/v) methanol, 7.5% (v/v) acetic acid. Molecular weight markers ranging from 42,700-200,000 (BDH Laboratory Supplies, Poole, England) were used as protein standards.

Western blot analysis

Proteins were transferred from a 12% slab gel (acrylamide:methylenebisacrylamide was 37.5:1) to a nitrocellulose membrane by the method of Towbin et al. (1979). After transfer, the membrane was incubated in a 5% bovine serum albumin-phosphate buffer solution (BSA-PBS) for 30 min at 37°C and then was washed three times in a 0.1% BSA-PBS solution for 5 min at room temperature. A monoclonal antibody (mAb) to desmin (D-1033) (Sigma, St. Louis, MO, USA) was used as a primary antibody. The membrane was incubated with the primary antibody for 16 h at 0°C, washed three times in 0.1% BSA-PBS for 5 min, incubated with immunogold-labeled secondary antibody for 2 h at room temperature, washed twice in 0.1% BSA-PBS solution for 5 min each and twice in deionized water for 1 min each. The gold label was enhanced by silver staining (Moeremans et al., 1989).

RESULTS AND DISCUSSION

Figure 1 showed that pH of breast (BM) samples from both Taiwan country chickens (TCC) and silkie bantam (SB) was significantly ($p<0.05$) lower than that of leg (LM) samples during postmortem storage at 5°C. This results were consistent the findings of Rose (1997), who stated that final pH was lower in chicken breasts (white) muscles than in leg (red) muscles, and also similar to the results of Christensen et al. (2004), who showed that the ultimate pH was lower in porcine white muscles than in red muscles. This difference could be attributed to the content of muscle glycogen predominantly stored in the white muscles (Schreurs, 2000) and to the difference in the metabolic properties between two types of muscles (Monin et al., 1987). However, pH was no difference ($p>0.05$) in same type of muscles between two avian strains (Figure 1).

Titin and nebulin were two giant proteins in skeletal

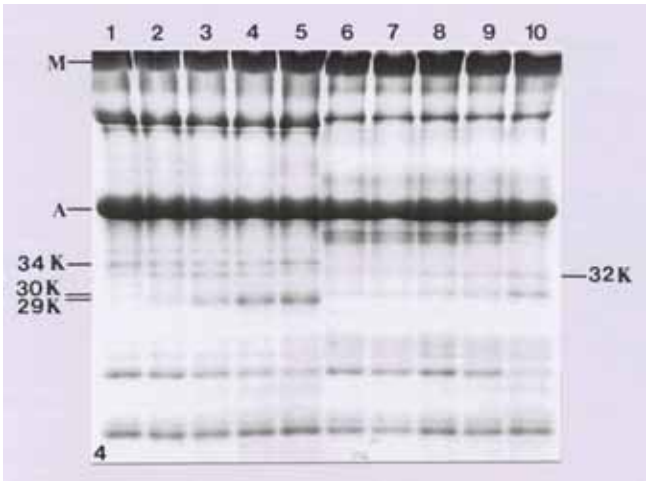

Figure 4. Changes in myofibrillar proteins of breast (BM) and leg (LM) muscles from Taiwan colored chickens. BM samples = lanes 1-5; LM samples = lanes 6-10; 0-day = lanes 1 and 6; 1-day = lanes 2 and 7; 3-day = lanes 3 and 8; 7-day = lanes 4 and 9; 14-day = lanes 5 and 10. M, myosin heavy chains; A, actin; 34 K, 34 kDa component; 32 K, 32 kDa component; 30 K, 30 kDa component; 29 K, 29 kDa component.

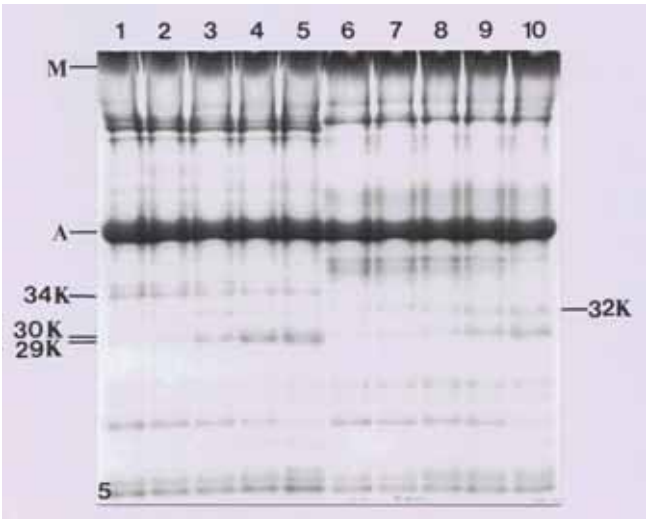

Figure 5. Changes in myofibrillar proteins of breast (BM) muscles and leg (LM) muscles from silkie bantam. BM samples = lanes 1-5; LM samples = lanes 6-10; 0-day = lanes 1 and 6; 1-day = lanes 2 and 7; 3-day = lanes 3 and 8; 7-day = lanes 4 and 9; 14-day = lanes 5 and 10. M, myosin heavy chains; A, actin; 34 K, 34 kDa component; 32 K, 32 kDa component; 30 K, 30 kDa component; 29 K, 29 kDa component.

muscles (Wang et al., 1979) and were present in a wide variety of animal species (Locker and Wild, 1986). According to our SDS-PAGE results, the migration of the titin and nebulin in the 0-day samples compared well with the typical electrophoretic patterns reported by Wang et al. (1979). Titin migrated near to the top of the gels as a closely spaced protein doublet (refer to titin 1 (T1) and titin 2 (T2)), and nebulin was clearly visible below titin (Wang et al., 1979). Our results showed that titin (arrow in Figures 2 and 3) and nebulin (arrowhead in Figures 2 and 3) in LM samples migrated slower than those in BM samples on the gels, in agreement with the results of Hu et al. (1986) and Tanabe et al. (1997). This difference might be due to the difference in the molecular weights of titin and nebulin between two types of muscles (Hu et al., 1986; Tanabe et al., 1997). Sorimachi et al. (1997) further reported that red and white muscles expressed different titin isoforms and that red muscles expressed the longer variant than that in white muscle.

Our SDS-PAGE results (Figures 2 and 3) also showed that the disappearance of the T1 occurred almost no obvious difference in both TCC and SB strains. The T1 band in BM samples was very faintly seen at day 0 and disappeared by day 1 (Figures 2 and 3). In LM samples, however, the T1 band was visible at day 0 and day 1 but disappeared by day 3 (Figures 2 and 3). These results also indicated that the disappearance of the T1 band occurred more rapidly in BM than in LM samples in both avian strains.

On the other hand, the disappearance of nebulin was slightly different between TCC and SB strains. The nebulin band in BM samples of TCC was faintly seen at day 0 and disappeared by day 1. In BM samples of SB, in contrast, the nebulin band remained visible by day 1 and disappeared by day 3. The nebulin band in LM samples of TCC was clearly visible at day 0, became faintly seen by day 1 and disappeared by day 3 (Figure 2). In LM samples of SB, however, the nebulin band was clearly visible at day 0 and day 1, became faintly seen by day 3 and disappeared by day 7 (Figure 3). These results showed that the disappearance of the nebulin band occurred slightly earlier in BM than in LM samples and also in TCC than in SB samples.

The main postmortem change in proteins migrating below myosin heavy chains was that the 29-35 kDa bands were present in both BM and LM samples of two avian strains as postmortem time proceeded (Figures 4 and 5). It was reported that that those ~30 kDa bands resulted from degradation of troponin-T in beef muscle (Ho et al., 1994; Muroya et al., 2003, 2004). Among those bands, the bands of 29 kDa and 34 kDa components in BM samples and the 30 kDa and 32 kDa components in LM samples were more apparent (Figures 4 and 5). Furthermore, the bands of 29 kDa component in BM samples and the 30 kDa component in LM samples became more prominent by day 14. This accumulation was more obvious in BM than in LM samples and was less in TCC than in SB samples (Figures 4 and 5).

Western blots labeled with a mAb (D-1033) to desmin (Figures 6a and 7a) demonstrated that intact desmin in BM samples of TCC and SB could be seen at day 0 and completely disappeared by day 1. In LM samples of both avian strains (Figures 6b and 7b), however, intact desmin seemed to remain no change until day 3 and began to

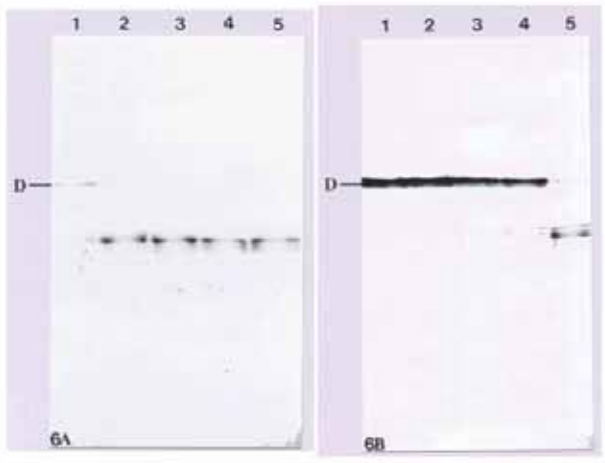

Figure 6. Western blots prepared from 12% gels of (A) breast and (B) leg muscle samples from Taiwan colored chickens were labeled with desmin monoclonal antibody. 0-day = lane 1; 1-day = lane 2; 3-day = lane 3; 7-day = lane 4; 14-day = lane 5.

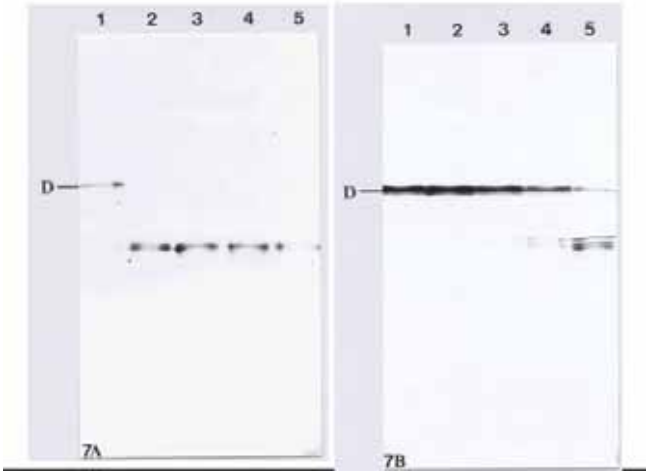

Figure 7. Western blots prepared from 12% gels of (A) breast and (B) leg muscle samples from silkie bantam were labeled with desmin monoclonal antibody. 0-day = lane 1; 1-day = lane 2; 3-day = lane 3; 7-day = lane 4; 14-day = lane 5.

degrade by day 7. By day 14, intact LM desmin degraded more in TCC samples than in SB samples.

Our results showed that accumulation of the ~30 kDa component and degradation of titin, nebulin and desmin occurred more rapidly in BM samples than in LM muscle in both strains, consistent with other reports (Samejima et al., 1976; Chou et al., 1994; Cha et al., 2001), and there was no significant difference between TCC and SB samples. Previous reports (Farouk et al., 1992) suggested that the accumulation of the ~30 kDa component could also be considered as an indicator of the rate of proteolysis. Therefore, the postmortem proteolysis at 5°C was more rapidly in BM than in LM samples. It was reported (Koohmaraie et al., 1988) that the extent of postmortem proteolysis in bovine white muscles was greater than that in bovine red muscles. The calpain enzyme system, especially μ-calpain, was considered as a key contributor in postmortem proteolysis of bovine muscles stored at 5°C (Koohmaraie et al., 1987; Boehm et al., 1998). In lamb muscles, in addition, activity of capastatin (calpain specific-inhibitor) was lower in white muscles than in red muscles. Furthermore, the ratio of μ-calpain:capastatin in porcine muscle was found higher in white muscles than in red muscles (Christensen et al., 2004). On the other hand, our results indicated that the pH of LM samples is more close to the optimal pH of calpain system (pH 7.2-7.0). It might imply that the extent of postmortem proteolysis in LM samples should be more extensive than that in BM samples. However, our present results did show that BM had more extensive postmortem proteolysis. Collectively, the difference in the extent of postmortem degradation of myofibrillar proteins between BM and LM muscles might depend more on the ratio of μ-calpain:capastatin than the pH effect in two types of muscles.

CONCLUSION

Postmortem proteolysis of breast (BM) and leg (LM) muscles from Taiwan colored chickens (TCC) and silkie bantam (SB) at 5°C were compared. Myofibrils were prepared from BM and LM samples that were randomly taken from the carcasses of SB and TCC after 0, 1, 3, 7 and 14 days of storage at 5°C. Our results showed that pH was lower in BM than in LM samples from both avian strains. Appearance of 30 kDa components and disappearance of titin and nebulin were more rapidly as seen on SDS-PAGE in BM than in LM samples. Western blots labeled with a monoclonal antibody to desmin also demonstrated that desmin degraded more quickly in BM samples. Our data might suggest that postmortem proteolysis occurred more rapidly in BM than in LM from both TCC and SB.

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